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Comparative Study of Pharmacokinetics and Serum Bactericidal Activity of Ceftizoxime and Cefotaxime

FRANÇOIS VALLÉE AND MARC LEBEL*

Laboratoire de Pharmacocinétique Clinique, École de Pharmacie, Université Laval, Cité Universitaire, Québec, Québec, Canada G1K 7P4

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Single 2-g intravenous doses of ceftizoxime (CZX) and cefotaxime (CTX) were given over 30 min to 10 adult volunteers in a crossover manner on two separate occasions. Concentrations of CZX, CTX, and the primary metabolite of CTX, desacetylcefotaxime (dCTX), in serum, suction-induced-blister fluid, and urine were determined by high-pressure liquid chromatography. Pharmacokinetic parameters were estimated by using an extended least-squares modeling program (MKMODEL). CZX exhibited a half-life in serum (2.05 h) longer than that of CTX (1.43 h) but comparable to that of dCTX (2.02 h). The percentage of penetration in blister fluid, estimated by area under the curve ratios, was significantly higher for CZX (164.4%) than for CTX (60.8%). Serum bactericidal activity, determined for volunteer samples at 1, 6, 8, and 12 h after patients were dosed, against clinical isolates of the *Bacteroides fragilis* group, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Morganella morganii* were significantly higher for CZX than those for CTX against members of the family *Enterobacteriaceae* at all times. Serum bactericidal titers against *B. fragilis* were also higher for CZX than for CTX at 1 h postinfusion. Neither CZX nor CTX exhibited any bactericidal activity at any other time against the *B. fragilis* group. In conclusion, the serum bactericidal activity of CZX was greater and more-prolonged than that of CTX against tested strains in spite of the in vitro synergistic contribution of dCTX to CTX, equal serum elimination half-lives of dCTX and CZX, and similar antibacterial activity and similar instability under microbiological testing for CZX and CTX.

Ceftizoxime (CZX) and cefotaxime (CTX) structural formulas differ only by an acetoxy group at position 3 on the beta-lactam ring. Although CTX is cleared more rapidly than CZX from the body, CTX is mainly metabolized in vivo to desacetylcefotaxime (dCTX), which also has intrinsic antibacterial activity; may act synergistically with CTX toward several pathogens (1, 7, 21, 24, 29, 32, 36); and possesses a half-life similar to that of CZX. On this basis, comparison of the pharmacokinetics and serum bactericidal activity of CZX and CTX is warranted.

MATERIALS AND METHODS

Healthy volunteers. Ten healthy volunteers (four women and six men) provided written informed consent for the study, which had been approved by the Centre Hospitalier de l'Université Laval Human Research Committee. The mean age of the volunteers was 25.1 years (range, 20 to 36 years), and the mean weight was 65.4 kg (range, 52.6 to 78 kg).

There was no history of hepatic, renal, or neoplastic disease or allergy to antimicrobial agents. A complete physical examination, hematology, blood chemistry, and urinalysis were performed for each subject before and after the study. All subjects were nonsmokers. Female volunteers were not pregnant according to direct latex agglutination pregnancy tests. Medication, alcohol, and beverages containing caffeine were withheld 24 h prior to and during the trial. Volunteers had to refrain from any strenuous or athletic activity during the study period but were allowed to circulate around the clinical pharmacokinetic unit.

Antibiotic administration. The compounds used in the study were CZX (Smith, Kline and French Laboratories, Philadelphia, Pa.) and CTX (Roussel Canada Inc., Montreal, Quebec). Each subject received a 2-g dose of CZX or CTX. Antibiotics were diluted in 40 ml of 5% glucose and given intravenously (i.v.) over a 30-min period in a crossover manner 1 week apart. Administration of these solutions into the antecubital vein via an i.v. cannula was performed by a constant infusion pump (Bard Canada Inc., Mississauga, Ontario).

Sampling. Blood samples were obtained from the cannula inserted in the contralateral arm at 0, 5, 15, 30, and 45 min and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h after the start of infusion. A diluted heparin solution (33 U/ml) was used to maintain patency of the needle, and at least 1 to 1.5 ml of blood was removed and discarded before blood for testing was drawn. Blood samples collected into Vacutainer tubes with no anticoagulant were allowed to clot at room temperature for 20 min and then were centrifuged (1,000 × *g*, 20 min, 4°C) to obtain serum.

Urine was collected immediately before cephalosporin administration and at intervals of 0 to 2, 2 to 4, 4 to 8, and 8 to 12 h thereafter. Urine volume and pH were recorded for each collection. Serum (5 ml) and urine (10 ml) samples were placed in polypropylene tubes and promptly frozen at −80°C until analysis.

Samples containing CTX were mixed with acetate buffer (final pH, 5.5) prior to freezing to minimize degradation (5). No hemolysis of the samples was noted; CTX hydrolysis occurs in the presence of hemolyzed blood (37).

Each volunteer had 10 skin blisters produced on the forearm by a slightly modified version of the technique of Hellum et al. (15, 30). Harvesting was at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 h. The samples were stored in polypropylene microtubes at −80°C until assayed.

Analytical procedure. Concentrations of antibiotics in serum, urine, and blister fluid were measured by a reverse-

* Corresponding author.

phase high-performance liquid chromatography (HPLC) procedure previously described (20) and adapted in our laboratory. The chromatographic system (Millipore-Waters, Bedford, Mass.), was equipped with a model 510 pump, a WISP model 710B automatic injector, a radial-compression separation system (Z-MODULE), a NOVA-PAK C_{18} (4-μm) cartridge, a Lambda-Max model 481 LC spectrophotometer, and a model 740 data module integrator.

The mobile phase consisted of 0.2 M sodium acetate and 0.2 M acetic acid in water-methanol (80:20). The flow rate was maintained at 1.5 ml/min, and the wavelength was set at 254 nm. Sample preparation involved internal standard (aminophylline) incorporation and protein precipitation with acetonitrile followed by delipidation with methylene chloride. The sensitivity limit of the assay in serum for any of these three compounds was 0.1 μg/ml. The day-to-day coefficients of variation were less than 6.1 for CZX, 4.4 for CTX, and 3.2% for dCTX for concentrations of 0.5, 5, and 50 μg/ml. Linear regression analysis of the standard curves yielded a correlation of ≥0.999, indicating an excellent linearity between 0.1 to 150, 0.1 to 200, and 0.1 to 30 μg/ml for CZX, CTX, and dCTX, respectively.

Pharmacokinetic analysis. Serum and blister fluid profiles for each volunteer were fitted to a sum of exponentials by using the iterative extended least-squares modeling program MKMODEL (16). The single variance model is provided by the equation $\mathrm{Var}(Y) = \mathrm{SD}^2 \cdot (\mathrm{VO} + Y^{\mathrm{PWR}})$, in which $\mathrm{Var}(Y)$ is the predicted variance, Y is the concentration predicted by a pharmacokinetic model, PWR is the power parameter, VO is the expected variance model when Y is zero and PWR is not zero, and SD is the standard deviation.

Pharmacokinetic parameters for all subjects for CTX and CZX in serum were described by a two-compartment model according to the following equation:

$$C = \left[\frac{R^0 \cdot (k_{21} - \alpha) \cdot (1 - e^{-\alpha \cdot t_i})}{V \cdot \alpha \cdot (\alpha - \beta)} \cdot e^{-\alpha \cdot t}\right] + \left[\frac{R^0 \cdot (\beta - k_{21}) \cdot (1 - e^{-\beta \cdot t_i})}{V \cdot \beta \cdot (\alpha - \beta)} \cdot e^{-\beta \cdot t}\right]$$

where C is the concentration in serum at time t, R^0 is the zero order infusion rate, α is the fast-disposition rate constant, β is the slow-disposition rate constant, k_{21} is the distribution rate constant from the central to the peripheral compartment, V is the volume of distribution, t_i is the infusion time, t is the total time since the start of infusion.

The area under the curve (AUC) from zero to infinity was calculated with a logarithmic trapezoidal rule and extrapolation methods from observed points. In turn, the AUC was used to evaluate total body clearance (dose/AUC_∞) and renal clearance ($A_{et_1-t_2}/\mathrm{AUC}_{t_1-t_2}$) where AUC_∞ is the AUC at infinity, $A_{et_1-t_2}$ is the amount of unchanged drug excreted into the urine during the time interval t_1 to t_2, and $\mathrm{AUC}_{t_1-t_2}$ is the AUC during the time interval t_1 to t_2. Nonrenal clearance was estimated by subtraction of renal clearance from total clearance. The percentage of doses excreted unchanged in the urine was calculated from the ratio (A_e/dose) × 100. The percentage of penetration into blister fluid was evaluated by the AUC ratios from 0 to t: $\mathrm{AUC}_{\mathrm{BF}}/\mathrm{AUC}_{\mathrm{Serum}}$, where $\mathrm{AUC}_{\mathrm{BF}}$ is the AUC for blister fluid.

Concerning dCTX in serum and blister fluid, elimination rate constants were calculated by linear regression of concentrations during terminal elimination phase. Renal clearance, AUC, fraction of metabolite excreted in urine, and percentage of penetration into blister fluid were calculated as for CTX.

Microbiological procedures. Serum inhibitory and bactericidal activities were determined by a National Committee for Clinical Laboratory Standards broth microdilution method (26). In order to comply with these guidelines, we tested 30 different batches of human serum to be used as diluents in assays for the absence of antimicrobial activity. MICs of CZX, CTX, and dCTX were determined in Mueller-Hinton broth supplemented or not with these 30 different batches of human serum against American Type Culture Collection control strains. These MICs with human serum-supplemented media did not fall in accepted ranges. Only horse serum (Flow Laboratories, Inc., McLean, Va.) did not show any antimicrobial effect. In tests with horse serum, the incubation medium used in our serum inhibitory and bactericidal activity determinations was 50% Mueller-Hinton broth and 50% horse serum (9).

To assess the potential influence of different protein binding of CZX, CTX, and dCTX in horse serum and human serum, we determined the protein binding of these three agents in horse serum with the drugs at concentrations of 10, 20, and 50 μg/ml by using equilibrium cell dialysis (12). Results showed protein binding to be higher for CZX and CTX in horse serum (61 and 62%, respectively) than in human serum (31 and 38%, respectively) (28, 33). dCTX exhibited similar binding in both kinds of serum. Considering that fractions of unbound CZX and CTX in horse serum were only half as large as those in human serum, we calculated that serum bactericidal titers could differ by only 1 dilution factor.

Mueller-Hinton broth was replaced by prereduced enriched thioglycolate broth (vitamin K, hemin) for anaerobic-microorganism determinations. Serum inhibitory and bactericidal activity determinations were performed at 1, 6, 8, and 12 h after the beginning of drug administration in order to assess peak and trough levels for these antibiotics. Serum samples were serially diluted in twofold steps from 1:2 to 1:2,048 with horse serum in 96-well microplates. Each test well contained 50 μl of an antibiotic dilution and 50 μl of the inoculum. The final inoculum size (10^5 to 10^6 CFU/ml) was verified by performing colony counts. Microplates containing aerobic strains were incubated for 18 h at 35°C, whereas anaerobic determinations were carried out with GasPak (BBL) anaerobic jars, which were incubated at 35°C for 24 h.

Four isolates of *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Morganella morganii* and five isolates of the *Bacteroides fragilis* group were tested against all volunteers. The bacteria were isolated from clinical material by the Department of Microbiology of the Centre Hospitalier de l'Université Laval.

Microbiological characterization of all strains was performed by determining MICs and MBCs against CZX, CTX, and dCTX by using the broth microdilution methods described by the National Committee for Clinical Laboratory Standards (25) and by Rosenblatt (34) for aerobic and anaerobic bacteria, respectively. Broths, inocula, and incubation conditions were as previously described. Quality control of antimicrobial susceptibility determinations was ensured by using *E. coli* ATCC 25922, *B. fragilis* ATCC 25285, and *Bacteroides thetaiotaomicron* ATCC 29741.

Interaction between CTX and its desacetyl metabolite in vitro has been studied with the 21 tested strains by using the checkerboard method (13). Both dCTX and CTX were tested alone and in combination (in a 1:1 ratio) by preparing serial

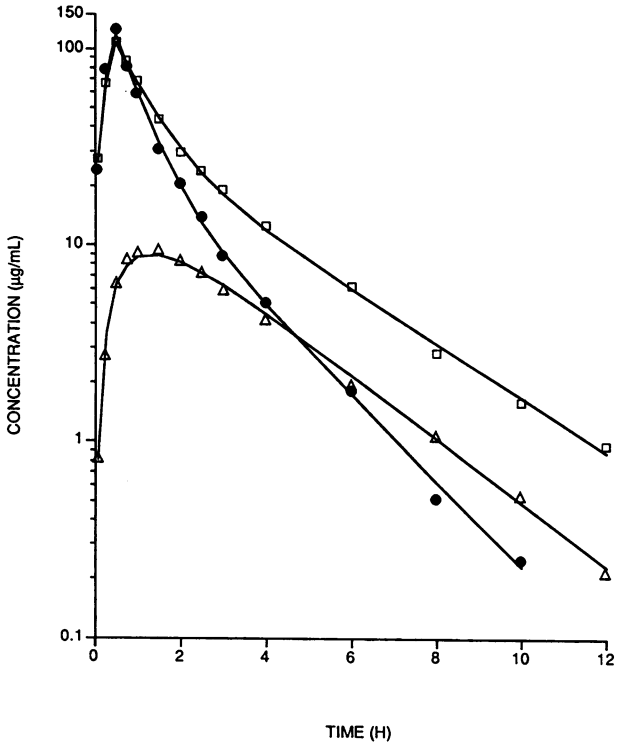

FIG. 1. Concentration-time curves for CZX, CTX, and dCTX in serum in 10 volunteers after a 2-g i.v. dose administered over 30 min. Symbols: ●, CTX; □, CZX; △, dCTX.

twofold dilutions in concentrations ranging from 4 to 5 dilutions below the MIC to three times the MIC. MICs were read 18 or 24 h later. Results were expressed as fractional inhibitory concentrations (FICs) and calculated from the following equation as suggested by Elion et al. (11): FIC = $(CTX_C/CTX_A) + (dCTX_C/dCTX_A)$, where CTX_A and $dCTX_A$ are the MICs obtained with CTX and dCTX alone and CTX_C and $dCTX_C$ are the concentrations of each compound in the lowest inhibitory combination. The FICs were interpreted as follows: ≤0.5, synergy; 0.51 to 0.75, partial synergy; and 0.76 to 2.0, additive (14).

Stability. This study also investigated the stability of CZX, CTX, and dCTX under HPLC analytical conditions and in in vitro microbiological tests. Stability during HPLC analysis in serum and acetate buffer (pH 5.5) was tested in duplicate experiments at ambient temperature (22°C) for up to 24 h. Two different concentrations of CTX (50 and 200 μg/ml) and dCTX (10 and 50 μg/ml) and one of CZX (10 μg/ml) in fresh human serum and acetate buffer were prepared and then analyzed every 15 min for drug remaining.

Determination of stability during microbiological tests was achieved by preparing solutions of CZX, CTX, and dCTX (10 μg/ml) in acetate buffer (pH 5.5), human serum, Mueller-Hinton broth, enriched thioglycolate broth, and a combination of serum and broths. Solutions containing thioglycolate were incubated anaerobically, and others were incubated aerobically. All were incubated for 24 h at 35°C. During the incubation period, samples were assayed in triplicate by HPLC every 6 h to evaluate the concentration of remaining drug. pH values were recorded at the end of each interval for each solution. All stability results were expressed as percentages of degradation and were calculated by the equation $1 - (C_t/C_0) \times 100$, where C_t is the residual concentration at time t and C_0 is the initial concentration.

Statistical analysis. Data on serum bactericidal activity are presented as geometric means. Otherwise, concentrations in serum, urine, and blister fluid and pharmacokinetic parameters are listed as means ± standard deviations. The Student paired t test was used for the determination of overall differences between treatments in the same subjects. The level of significance for all statistical tests was set at $P < 0.05$.

RESULTS

Pharmacokinetics. The concentration-time curves of CZX, CTX, and dCTX in serum following a single 2-g intravenous dose are shown in Fig. 1. Corresponding pharmacokinetic parameters are given in Table 1. Maximum levels in serum (at the end of a 30-min infusion) of CZX (114.4 μg/ml) and CTX (125.9 μg/ml) were similar. After 12 h, it was still possible to detect CZX (1.0 μg/ml), but amounts of CTX, if it was present, were below the detection limit. dCTX formation was rapid: the peak concentration (10.1 μg/ml) was reached between 30 and 40 min after parent drug administration. Along the serum dCTX concentration-time curve, the ratios of dCTX to CTX increased continuously; the ratio equalled unity after 4 h and was slightly over 2 between 8 and 10 h.

A comparison of the pharmacokinetic parameters of CZX and CTX revealed statistically significant differences. CZX exhibited an elimination half-life (2.05 h) longer than that of CTX (1.43 h) but comparable to that of dCTX (2.02 h). Total body clearance (3.82 versus 2.76 ml/min/kg of body weight) and nonrenal clearance (2.29 versus 0.78 ml/min/kg) were significantly faster for CTX. AUC values were significantly higher for CZX (192.5 versus 141.9 μg/h/ml), whereas differences in volume of distribution and renal clearance were not significant. The cumulative urinary excretion of intact drugs

TABLE 1. Pharmacokinetic parameters of CZX, CTX, and dCTX in serum[a]

Drug	C_{max} (μg/ml)	C_{8h} (μg/ml)	$t_{1/2\beta}$ (h)	AUC (μg · h/ml)	V (liters/kg)	CL (ml/min/kg)	CL_R (ml/min/kg)	CL_{NR} (ml/min/kg)	F_u (%)
CZX	114.37 ± 30.51	2.83 ± 1.46[b]	2.05 ± 0.44[c]	192.52 ± 40.21[c]	0.34 ± 0.10	2.76 ± 0.78[b]	1.98 ± 0.45	0.78 ± 0.44[b]	72.88 ± 9.32[b]
CTX	125.86 ± 19.18	0.51 ± 0.72	1.43 ± 0.38	141.93 ± 30.77	0.28 ± 0.07	3.82 ± 1.02	1.53 ± 0.34	2.29 ± 0.89	41.45 ± 8.65
dCTX	10.05 ± 1.77	1.09 ± 0.60	2.02 ± 0.58	39.06 ± 7.36			96.82 ± 28.23		19.76 ± 3.45

[a] Data are given as mean values ± standard deviations for 10 volunteers. Abbreviations: C_{max}, maximum concentration of drug in serum; C_{8h}, concentration of drug in serum at 8 h; $t_{1/2\beta}$, elimination half-life; V, volume of distribution; CL, total clearance; CL_R, renal clearance; CL_{NR}, nonrenal clearance; F_u, excretion of unchanged drug in urine.

[b] $P < 0.01$.

[c] $P < 0.05$.

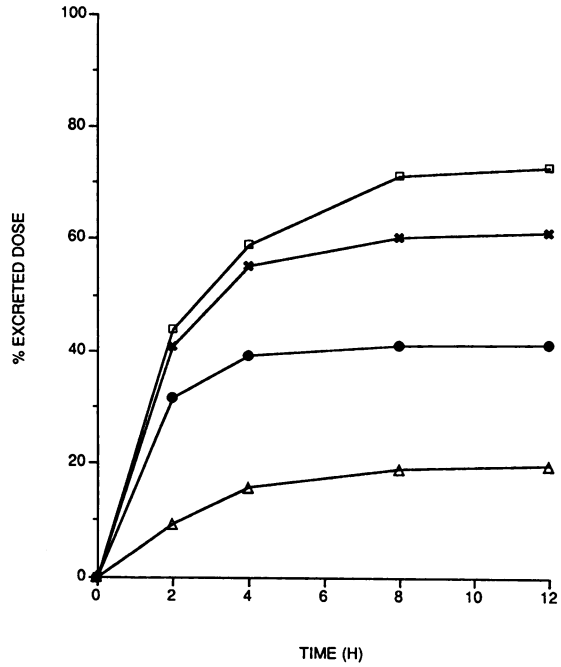

FIG. 2. Excretion of CZX, CTX, and dCTX in urine of 10 volunteers after a 2-g i.v. dose administered over 30 min. Symbols: ●, CTX; □, CZX; △, dCTX; ×, CTX and dCTX.

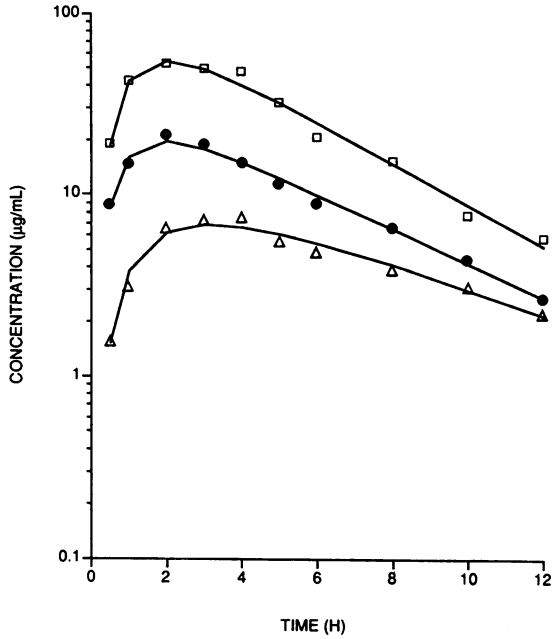

FIG. 3. Concentration-time curves for CZX, CTX, and dCTX in suction-induced-blister fluid in 10 volunteers after a 2-g i.v. dose administered over 30 min. Symbols: ●, CTX; □, CZX; △, dCTX.

and metabolite is illustrated in Fig. 2. The 12-h urinary recovery rates of CZX and CTX were 72.9 and 41.5%, respectively ($P = 0.0001$). Urinary excretion of dCTX accounted for 19.8% of the administered dose of CTX.

Besides CTX and dCTX, a third compound was observed on chromatograms of urine. This unidentified compound probably corresponds to a dCTX metabolite (M2 or M3). No further analysis of this observation was performed.

Concentration-time profiles of CZX, CTX, and dCTX in blister fluid are shown in Fig. 3, and corresponding pharmacokinetic parameters are given in Table 2. Compared with CTX, CZX yielded significantly higher peak levels (58.2 versus 24.3 μg/ml). Half-lives in blister fluid were very similar for both drugs (2.9 h) but were 1.4 to 2 times longer than in serum. The percentage of penetration in suction-induced blister fluid, as estimated by the AUC ratios, was significantly higher for CZX (164.4%) than for CTX (60.8%).

The maximum level of dCTX in blister fluid (8.4 μg/ml) was comparable to that in serum (10.1 μg/ml). The half-life of dCTX was two times longer in blister fluid than in serum. The ratio of the AUC of dCTX in blister fluid to that in serum (135.8%) was 2.2 times greater than the same ratio for CTX.

MIC-MBC determinations. Table 3 lists the MICs and MBCs of CZX, CTX, and dCTX. Susceptibility of members of the family *Enterobacteriaceae* to CZX and CTX varied from highly susceptible (MIC ≤ 0.06 μg/ml) to moderately susceptible (MIC ≤ 16 μg/ml). CTX was slightly more active than CZX against *E. cloacae* and *M. morganii*. When the two cephalosporins were tested against *E. coli* and *K. pneumoniae*, values were comparable. Susceptibility of members of the *B. fragilis* group to CTX and CZX ranged from 16 to 64 μg/ml. CTX MICs and MBCs were up to fourfold greater than those of CZX against the same anaerobic strains. The antibacterial activity of dCTX was 2- to 64-fold lower than that of CTX against *Enterobacteriaceae*. dCTX is slightly less active than its precursor against the *B. fragilis* group.

Serum bactericidal activity. Serum bactericidal activities of CZX and CTX at 1, 6, 8, and 12 h after infusion and corresponding levels of the drugs in serum are summarized in Table 4. Data are expressed as geometric means of reciprocal serum bactericidal titers. Peak bactericidal activity (1 h) in serum containing CZX or CTX achieved titers of 1:215 or greater for all *Enterobacteriaceae*. At 6, 8, and 12 h, CZX was significantly more bactericidal than CTX ($P < 0.001$) against *E. cloacae*, *E. coli*, *K. pneumoniae*, and *M. morganii*. Twelve hours after CZX administration, significant bactericidal levels against all *Enterobacteriaceae* were

TABLE 2. Pharmacokinetic parameters of CZX, CTX, and dCTX in suction-induced-blister fluid[a]

Drug	C_{max} (μg/ml)	C_{8h} (μg/ml)	$t_{1/2\beta}$ (h)	AUC (μg · h/ml)	Penetration (%)
CZX	58.21 ± 15.06[b]	15.37 ± 4.96[b]	2.89 ± 0.78	323.24 ± 54.19[b]	164.38 ± 87.39[b]
CTX	24.28 ± 6.48	6.63 ± 3.04	2.96 ± 0.76	124.35 ± 24.79	60.82 ± 16.27
dCTX	8.43 ± 1.37	3.93 ± 1.23	4.33 ± 1.60	70.90 ± 30.23	135.76 ± 36.31

[a] Data are given as mean values ± standard deviations for 10 healthy volunteers.
[b] $P < 0.01$.

TABLE 3. Antimicrobial activity of CZX, CTX, and dCTX against 21 gram-negative bacteria

Bacterium	CZX		CTX		dCTX	
	MIC	MBC	MIC	MBC	MIC	MBC
B. thetaiotaomicron A-20	16	32	64	64	64	64
B. fragilis A-35	8	16	64	64	64	64
B. fragilis A-89	8	16	64	64	128	128
B. thetaiotaomicron M-19[a]	16	16	16	16	128	128
B. thetaiotaomicron W-15	32	64	16	16	32	32
E. cloacae 1	2	4	0.5	1	32	64
E. cloacae 2	4	8	2	4	64	64
E. cloacae 3	0.25	0.5	0.12	0.25	8	16
E. cloacae 4[a]	0.25	0.25	0.25	0.25	4	4
E. coli 2	4	8	2	2	64	64
E. coli 351	8	8	8	8	>64	>64
E. coli 519[a]	0.12	0.12	0.12	0.12	0.5	0.5
E. coli 623	<0.06	<0.06	0.12	0.12	0.5	0.5
K. pneumoniae 1[a]	<0.06	0.12	<0.06	0.12	0.25	0.25
K. pneumoniae 2	<0.06	<0.06	<0.06	<0.06	0.25	0.25
K. pneumoniae K-294	4	4	<0.06	<0.06	0.12	0.12
K. pneumoniae K-308	<0.06	0.12	<0.06	<0.06	0.12	0.25
M. morganii 1	8	16	2	8	>64	>64
M. morganii 2	0.12	0.12	<0.06	<0.06	2	2
M. morganii 221[a]	<0.06	<0.06	<0.06	<0.06	0.5	0.5
M. morganii 233	4	8	0.5	0.5	32	32

[a] MBCs of CZX and CTX are identical.

noted. In contrast, serum bactericidal levels (1:2) for CTX could be seen only against *K. pneumoniae* at 12 h.

Bactericidal titers against the *B. fragilis* group at 1 h postinfusion were higher for CZX than for CTX ($P = 0.001$). Neither CZX nor CTX exhibited any bactericidal activity against the *B. fragilis* group at any other time. However, a serum inhibitory titer of 2 was noted at 6 h with CZX.

Checkerboard. Interactions between CTX and dCTX as interpreted by the FIC index are shown in Table 5. Synergistic effects against two strains of *E. coli*, two strains of *K. pneumoniae*, and one strain of *E. cloacae* were found. Partial synergy against most of the other strains (14 of 21) was demonstrated. An additive effect against two isolates of *B. thetaiotaomicron* was observed.

Stability. Table 6 displays mean percentages of degradation of CZX, CTX, and dCTX after 18 and 24 h under chromatographic and microbiological conditions in various media. These cephalosporins showed a small percentage of degradation (≤4.3%) under chromatographic conditions except for CTX in serum, which had a loss of approximately 11% after 24 h at room temperature. This observation was taken into consideration in reporting concentrations of CTX in serum.

The in vitro stability of these cephalosporins was more affected under microbiological conditions than under chromatographic conditions. Concentrations decreased over the incubation period in all tested media, and the greatest declines were always observed with CTX. The highest degradation occurred in serum, with a loss of 55% for CTX, 33% for CZX, and 20% for dCTX after 24 h of incubation. Levels of CTX and dCTX were respectively reduced by 35 and 18% in serum containing Mueller-Hinton or thioglycolate broth. In contrast, Mueller-Hinton broth alone or in combination with serum produced a greater reduction in CZX concentrations (24 to 29%) than did thioglycolate (15%). Degradation of CZX and CTX was also found in broth alone and in acetate buffer, but to a lesser extent (30 to 5%).

Adverse reactions. During the study period and only after CTX administration, four volunteers developed headaches that started about 1 h after infusion. Skin rash was observed

TABLE 4. Comparison of serum bactericidal activities of CZX and CTX

Bacterium	SBT at[a]:							
	1 h		6 h		8 h		12 h	
	CTX (59.72[b])	CZX (68.63)	CTX (1.82)	CZX (6.12[c])	CTX (0.51)	CZX (2.83[c])	CTX (0)	CZX (0.9[c])
B. fragilis	2	10[c]	<2	<2	<2	<2	<2	<2
E. cloacae	215	239[c]	3	20[c]	2	9[d]	<2	4[c]
E. coli	321	261	3	17[c]	2	13[c]	<2	5[c]
K. pneumoniae	1,098	1,978[c]	16	201[c]	5	140[c]	2	41[c]
M. morganii	724	832[d]	11	62[c]	4	21[c]	<2	6[c]

[a] Data are expressed as the geometric means of reciprocal serum bactericidal titers (SBTs). Levels of dCTX were 9.21 μg/ml at 1 h, 1.96 μg/ml at 6 h, 1.09 μg/ml at 8 h, and 0.22 μg/ml at 12 h.

[b] Level in serum (micrograms per milliliter).

[c] $P < 0.01$.

[d] $P < 0.05$.

TABLE 5. Interaction of CTX and dCTX against gram-negative bacteria

Bacterium	Total no. of strains	No. of strains in which drugs showed:		
		Synergy	Partial synergy	Addition
B. fragilis group	5		3	2
E. cloacae	4	1	3	
E. coli	4	2	2	
K. pneumoniae	4	2	2	
M. morganii	4		4	

in one subject 3 h postdose, and two volunteers complained of diarrhea.

DISCUSSION

Several pharmacokinetic and antibacterial-activity aspects of CZX, CTX, and dCTX have been reported separately, although few investigations have provided comparative analysis by using the same population and the same analytical conditions (6, 18, 31, 34). Our study compared the pharmacokinetics of CZX and CTX by using a crossover design and an HPLC assay that accurately measures CZX, CTX, and dCTX at concentrations as low as 0.1 μg/ml. Levels in serum reached after i.v. administration of 2 g of CZX or CTX and pharmacokinetic parameters were usually similar to those found in other studies (8, 10, 27, 33). In contrast, CTX elimination half-life and blister fluid penetration by CZX and dCTX were slightly superior to those in the references cited. Longer half-life of CTX in serum in our subjects (1.43 h) than had been previously reported (0.7 to 1.3 h) can be attributed to the higher sensitivity of the HPLC assay used in this study and the consequently greater number of datum points available for the terminal elimination phase. The detection limit of the assay in most pharmacokinetic studies of CTX was 0.5 μg/ml, with detectable levels in serum for up to 6 or 8 h. We were able to determine concentrations of CTX in serum at 10 h postinfusion, with a 0.1-μg/ml sensitivity limit. Lüthy et al. (22) clearly demonstrated an increased half-life for CTX when a longer sampling period is used for calculation.

The results for blister fluid penetration by CTX (60.8%) are in good agreement with those of previously published reports (4, 32). AUC ratios of dCTX (135.8%) were twice those of CTX as described by Bergan et al. (4). In contrast, the penetration ratio of CZX (164.4%) was higher than those reported by Shyu et al. (35) and Korting et al. (19), i.e., 60 and 101%, respectively. The shorter blister fluid-sampling periods in those investigations (0 to 6 h [35] and 3 to 9 h [19]) compared with those of this study (0 to 12 h) may help explain the imprecise estimates of AUC for blister fluid by Shyu et al. (35) and Korting et al. (19), which in turn resulted in underestimations of the percentage of penetration by CZX.

That blister fluid/serum AUC ratios were higher for CZX and dCTX than for CTX may be attributed to the smaller molecular sizes and longer half-lives in serum of CZX and dCTX. These properties allow easier diffusion and maintenance of a prolonged high-concentration gradient between the vascular and extravascular compartments.

Like other investigators, we have demonstrated an in vitro synergistic interaction of CTX and dCTX against aerobic and anaerobic gram-negative bacteria (1, 7, 17, 21, 29, 36). These investigations showed considerable variability in the interaction of CTX and dCTX against the various bacteria tested. Synergistic activity against *Enterobacteriaceae* has been demonstrated in 40 to 78% of isolates (17, 21, 29). Despite the small number of clinical isolates in this study, results are in accordance with those previously reported. Complete or partial synergy against the *B. fragilis* group was reported for 40 to 90% of the test organisms, a situation apparently explained by regional differences in susceptibility patterns (1, 7, 36). Recent data showed that differences in anaerobic susceptibility determination techniques may better explain these discrepancies (2).

Serum bactericidal activity data showed that CZX produced significantly higher bactericidal activity than CTX and dCTX at any time against strains of *Enterobacteriaceae*. Except for *K. pneumoniae* isolates, bactericidal activity was present for up to 8 h with CTX and up to 12 h with CZX. Barriere et al. (3) and Bornstein (5a) have also demonstrated that CZX exhibited higher and more-prolonged bactericidal titers than CTX against *Enterobacteriaceae*. In contrast to those findings, Quintiliani et al. (31) reported similar bactericidal and bacteriostatic activities for both CZX and CTX over a 10-h period against a single strain each of *E. coli* and *B. fragilis*. In their study, mock serum samples were used and in vitro synergy with the combination of CTX and dCTX was observed only for the *B. fragilis* isolate.

In this study, the presence of dCTX did not extend the antimicrobial activity of CTX to produce titers comparable to those of CZX. The contribution of dCTX to the serum bactericidal activity of CTX, usually observed at the end of a dosing interval, when dCTX concentrations reached or

TABLE 6. Stability of CZX, CTX, and dCTX under microbiological and chromatographic conditions

Conditions	% Degradation of:											
	CZX at:				CTX at:				dCTX at:			
	6 h	12 h	18 h	24 h	6 h	12 h	18 h	24 h	6 h	12 h	18 h	24 h
Microbiological												
Acetate buffer	<1	<1	2.6	5.5	<1	3	13.3	19.9	2.8	5.7	9.9	15.1
Serum	12.6	19.8	26.2	33.4	15.2	29.4	45	55.2	8.6	14.8	17.5	20.6
Mueller-Hinton broth	6.3	11.6	17.6	29.2	11.1	21.5	27.6	29.9	10.5	12.4	13.4	14.2
Mueller-Hinton broth + serum	8.4	16.1	19.8	24.2	12.6	23.4	32.3	35.6	8.6	13.2	15.9	18.7
Thioglycolate[a]	3.8	6.6	9.9	15.2	10.5	22.4	26.6	28.9	3.4	7.8	11.2	14
Thioglycolate + serum[a]	2.6	10.5	13.2	15.3	7.3	25.3	32.1	34.5	5.5	8.2	13.3	17.2
Chromatographic												
Acetate buffer	<1	<1	<1	<1	<1	3.1	3.5	4.3	<1	<1	1.6	2.1
Serum	<1	<1	1.2	1.9	<1	4.7	9.3	11.2	2.7	3	3.4	4.2

[a] Medium incubated under anaerobiosis.

TABLE 7. Reassessment of serum bactericidal activity against bacteria exhibiting identical MBCs of CZX and CTX

Bacterium	SBT at[a]:							
	1 h		6 h		8 h		12 h	
	CTX (59.72[b])	CZX (68.63)	CTX (1.82)	CZX (6.12[c])	CTX (0.51)	CZX (2.83[c])	CTX (0)	CZX (0.9[c])
B. fragilis M-19	3	8	<2	2[d]	<2	<2	<2	<2
E. cloacae 4	630	1,552[d]	5	104[d]	3	49[d]	2	9[c]
E. coli 519	2,048	2,048	5	208[c]	3	158[c]	<2	39[c]
K. pneumoniae 1	1,176	2,048	15	208[c]	4	181[c]	<2	49[d]
M. morganii 221	1,783	2,048	23	208[c]	7	48[c]	<2	14[c]

[a] Data are expressed as the geometric means of reciprocal serum bactericidal titers (SBTs). Levels of dCTX were as given in Table 4, footnote *a*.
[b] Level in serum (micrograms per milliliter).
[c] $P < 0.01$.
[d] $P < 0.05$.

exceeded those of CTX (3, 24, 32), was minimal in our study and may be attributable to the high dCTX MBCs of the strains tested.

To better define the contribution of dCTX, we reassessed the comparison of bactericidal activity for CZX and CTX by selecting five strains (one of each genus) exhibiting identical MBCs to CZX and CTX and for which the combination of CTX and dCTX acted synergistically. Results displayed in Table 7 indicate that in spite of synergistic activity observed with the in vitro checkerboard technique and identical MBCs, our conclusions hold true: CZX bactericidal activity is superior to that of CTX.

Serum may be responsible for increasing the antibacterial activities of cephalosporins, but it could also contribute to their degradation. We have demonstrated the instability of CZX, CTX, and dCTX under conditions of in vitro microbiological testing. Serum components and elevated temperature seem to be the major factors affecting the stability of these antibiotics. There was no apparent correlation between pH and degradation of CZX, CTX, and dCTX. MIC test simulations (antibiotics in broths) and serum inhibitory titer simulations (antibiotics in broths and serum) revealed similar percentages of degradation (29 to 36%) for CZX and CTX over a 24-h incubation period. These results are consistent with those of the earlier work of Marchbanks et al. with CTX and dCTX (23). In contrast, CZX was more stable than CTX in media containing thioglycolate. This finding cannot be readily explained by the effect of anaerobiosis or the medium itself, since we found higher percentages of degradation with CTX under the same conditions. The greater decline observed in CTX concentrations in media containing serum may be attributed to CTX metabolism by serum esterases that resulted in deacetylation of CTX. Welch and Bawdon (37) have demonstrated that a reduction of 13 and 41% in CTX concentrations was noted in the presence of 1 and 10% hemolyzed blood, respectively. In spite of similar CZX and CTX degradation during in vitro microbiological testing, these results may lead to an underestimation of the in vivo activities of these drugs.

In summary, this study clearly demonstrated the superiority of CZX serum bactericidal activity over that of CTX and a similar degradation pattern for the two drugs under in vitro microbiological testing. Although dCTX possesses a serum elimination half-life similar to that of CZX, in vitro synergistic activity with CTX against tested strains, and a degree of bactericidal activity similar to that of the parent drug at the end of a dosing interval, it cannot extend the activity of CTX to produce bactericidal titers comparable to those of CZX.

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REFERENCES

1. **Aldridge, K. E., L. S. Weeks, C. W. Stratton, and C. V. Sanders.** 1989. Comparison of bactericidal activity of cefotaxime and desacetylcefotaxime alone and in combination against *Bacteroides fragilis* group organisms. Diagn. Microbiol. Infect. Dis. **12**:165–170.
2. **Aldridge, K. E., H. M. Wexler, C. V. Sanders, and S. M. Finegold.** 1990. Comparison of in vitro antibiograms of *Bacteroides fragilis* group isolates: differences in resistance rates in two institutions because of differences in susceptibility testing methodology. Antimicrob. Agents Chemother. **34**:179–181.
3. **Barriere, S. L., D. C. Ozasa, and J. Mordenti.** 1985. Assessment of serum bactericidal activity after administration of cefoperazone, cefotaxime, ceftizoxime, and moxalactam to healthy subjects. Antimicrob. Agents Chemother. **28**:55–57.
4. **Bergan, T., T. Kalager, K. B. Hellum, and C. O. Solberg.** 1982. Penetration of cefotaxime and desacetylcefotaxime into skin blister fluid. J. Antimicrob. Chemother. **10**:193–196.
5. **Berge, S. M., N. L. Henderson, and M. J. Frank.** 1983. Kinetics and mechanism of degradation of cefotaxime sodium in aqueous solution. J. Pharm. Sci. **72**:59–63.

5a. **Bornstein, D. L.** 1988. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 766.

6. **Burckart, G. J., R. J. Ptachcinski, D. H. Jones, D. L. Howrie, R. Venkataramanan, and T. E. Starzl.** 1987. Impaired clearance of ceftizoxime and cefotaxime after orthotopic liver transplantation. Antimicrob. Agents Chemother. **31**:323–324.
7. **Canawati, H. N.** 1989. Comparative in vitro activity of cefoxitin, cefotaxime alone, and in combination with desacetylcefotaxime against the *Bacteroides* species. Diagn. Microbiol. Infect. Dis. **12**:33–37.
8. **Carmine, A. A., R. N. Brogden, R. C. Heel, T. M. Speight, and G. S. Avery.** 1983. Cefotaxime: a review of its antibacterial activity, pharmacological properties and therapeutic use. Drugs **25**:223–289.
9. **Courvalin, P., F. Goldstein, A. Philippon, and J. Sirot.** 1985. Pouvoir bactériostatique et bactéricide d'un liquide biologique, p. 219–225. *In* L'antibiogramme, 1st ed. MPC Videom, Paris, France.
10. **Dudley, M. N., and S. L. Barriere.** 1982. Cefotaxime: microbiology, pharmacology, and clinical use. Clin. Pharm. **1**:114–124.
11. **Elion, G. B., S. Singer, and G. H. Hichings.** 1954. Antagonists of nucleic acid derivatives. VIII. Synergism in combinations of biochemically related antimetabolites. J. Biol. Chem. **208**:477–488.
12. **Fiset, C., F. Vallée, M. LeBel, and M. G. Bergeron.** 1986. Protein binding of ceftriaxone: comparison of three techniques of determination and the effect of 2-hydroxybenzoylglycine, a drug inhibitor in uremia. Ther. Drug Monit. **8**:483–489.
13. **Garrold, L. P., and P. M. Waterworth.** 1962. Methods of testing

antibiotic bactericidal action and the significance of the results. J. Clin. Pathol. **15**:328–338.
14. **Hall, M. J., R. F. Middleton, and D. Westmacott.** 1983. The fractional inhibitory concentration (FIC) index as a measure of synergy. J. Antimicrob. Chemother. **11**:427.
15. **Hellum, K. B., A. Schreiner, A. Digranes, and I. Bergman.** 1978. Skin blisters produced by suction: a new model for studies of penetration of antibiotics in humans, p. 620–622. *In* W. Siegenthaler and R. Luthy (ed.), Current chemotherapy, vol. 1. American Society for Microbiology, Washington, D.C.
16. **Holford, N.** 1987. MK model; an extended least squares modelling program. Version 3.34. Elsevier-Biosoft, Cambridge, United Kingdom.
17. **Jenkins, S. G.** 1989. Activity of cefotaxime/desacetylcefotaxime with two aminoglycosides against gram-negative pathogens: an example of interactive synergy. Diagn. Microbiol. Infect. Dis. **12**:51–55.
18. **Jordan, N. S., W. C. Shyu, R. Quintiliani, and C. H. Nightingale.** 1986. Comparative pharmacokinetics of ceftizoxime and cefotaxime in healthy volunteers. Ther. Res. **40**:134–140.
19. **Korting, H. C., M. Shäfer-Korting, R. Haag, and E. Mutschler.** 1984. Plasma, cantharides blister fluid, and suction blister fluid levels of ceftizoxime after single intramuscular application for gonorrhea. Int. J. Clin. Pharm. Ther. Toxicol. **22**:218–220.
20. **LeBel, M., J. F. Ericson, and D. H. Pitkin.** 1984. Improved high-performance liquid chromatographic (HPLC) assay method for ceftizoxime. J. Liq. Chromatogr. **7**:961–968.
21. **Limbert, M., G. Seibert, and E. Schrinner.** 1982. The cooperation of cefotaxime and desacetyl-cefotaxime with respect to antibacterial activity and β-lactamase stability. Infection **10**:97–100.
22. **Lüthy, R., J. Blaser, A. Bonetti, H. Simmen, R. Wise, and W. Siegenthaler.** 1981. Comparative multiple-dose pharmacokinetics of cefotaxime, moxalactam, and ceftazidime. Antimicrob. Agents Chemother. **20**:567–575.
23. **Marchbanks, C. R., R. L. Yost, and R. L. White.** 1987. Cefotaxime stability during in vitro microbiological testing. Antimicrob. Agents Chemother. **31**:1375–1378.
24. **Moore, E. S., M. Jimenez, S. L. Barriere, M. L. Cimino, and F. R. Fekety.** 1988. Additive and synergistic bactericidal activity contributed by desacetylcefotaxime during cefotaxime therapy. Clin. Pharm. **7**:901–905.
25. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
26. **National Committee for Clinical Laboratory Standards.** 1987. Methodology for the serum bactericidal test. Proposed guideline M21-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
27. **Neu, H. C.** 1984. Ceftizoxime: a β-lactamase-stable, broad spectrum cephalosporin. Pharmacotherapy **4**:47–60.
28. **Neu, H. C., N. Aswapokee, P. Aswapokee, and K. P. Fu.** 1979. HR 756, a new cephalosporin active against gram-positive and gram-negative aerobic and anaerobic bacteria. Antimicrob Agents Chemother. **15**:273–281.
29. **Oizumi, K., I. Hayashi, S. Aonuma, and K. Konno.** 1988. In vitro activity of desacetylcefotaxime and the interaction with its parent compound, cefotaxime. Drugs **35**(Suppl. 2):57–61.
30. **Panneton, A.-C., M. G. Bergeron, and M. LeBel.** 1988. Pharmacokinetics and tissue penetration of fleroxacin after single and multiple 400- and 800-mg-dosage regimens. Antimicrob. Agents Chemother. **32**:1515–1520.
31. **Quintiliani, R., C. H. Nightingale, and R. Tilton.** 1984. Comparative pharmacokinetics of cefotaxime and ceftizoxime and the role of desacetylcefotaxime in the antibacterial activity of cefotaxime. Diagn. Microbiol. Infect. Dis. **2**:63S–70S.
32. **Reller, L. B.** 1984. Interaction of cefotaxime and desacetylcefotaxime against pathogenic bacteria. Diagn. Microbiol. Infect. Dis. **2**:55S–61S.
33. **Richards, D. M., and R. C. Heel.** 1985. Ceftizoxime: a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs **29**:281–329.
34. **Rosenblatt, J. E.** 1984. Antimicrobial susceptibility testing of anaerobic bacteria. Rev. Infect. Dis. **6**(Suppl. 1):S242–S248.
35. **Shyu, W. C., R. Quintiliani, and C. H. Nightingale.** 1985. An improved method to determine interstitial fluid pharmacokinetics. J. Infect. Dis. **152**:1328–1331.
36. **Wasilauskas, B. L.** 1989. Effectiveness of cefotaxime alone and in combination with desacetylcefotaxime against *Bacteroides fragilis*. Diagn. Microbiol. Infect. Dis. **12**:39–43.
37. **Welch, W. D., and R. E. Bawdon.** 1986. Cefotaxime metabolism by hemolyzed blood: quantitation and inhibition of the deacetylation reaction. Diagn. Microbiol. Infect. Dis. **4**:119–124.